Contraction of Dried Casein Particle Surfaces Effected by Sequential H_2O Vapor Sorption and Desorption Cycles

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When casein micelles, isolated from milk by high-speed centrifugation, are washed with water and dehydrated by serial transfer through liquids of decreasing polarity before final solvent removal under vacuum, the resulting dry material has a specific surface area 10 times higher than washed micelles dried directly from aqueous systems by lyophilization. Subjecting the dried proteinaceous material to cyclic H_2O vapor sorption and desorption resulted in contraction and loss in BET surface area as measured by low temperature N_2 adsorption. The observed surface area decrease is proportional to the amount of water sorbed by the casein before removal. The data indicate that the micelles in milk may be subject to shrinkage and loss of porosity during drying by procedures based on direct water vapor transfer.

INTRODUCTION

Discrepancies in surface area values for lyophilized proteins determined from N_2 or H_2O adsorption data were reported as early as 1944 by Shaw (1). Theories advanced to explain these differences were based on the involvement of specific H_2O binding sites in the protein (2, 3) with the result that water vapor sorption by proteins is a bulk rather than a surface phenomenon (4). It has also been pointed out (5) that dehydrated macromolecules of biological origin such as proteins, starches, or celluloses may swell upon sorbing H_2O. With reference to their studies of H_2O sorption by mucopolysaccharides Bettelheim and Ehrlich (6) have stated that such swelling renders even the meaning of a surface area from H_2O adsorption data questionable.

In experiments with bacterial spores, Neihof, Thompson, and Dietz (7) demonstrated a loss in N_2 surface area as a result of lyophilization. They showed that lyophilized spores swell when put into aqueous suspension; but when these spores are dried by passage through a series of solvents of decreasing polarity a large surface is available for N_2 adsorption. Rehumidifying the spores at 87% to 93% relative humidity and desorbing H_2O under vacuum or rewetting with liquid H_2O and freeze drying led to collapse of the swollen hydrophilic structures with a concomitant loss in surface area. In an earlier study Merchant (8) reported similar results with cellulose fibers.

In the current investigation it was sought to demonstrate a similar swelling phenomenon in a protein system and to study the collapse of the swollen or expanded structure through H_2O sorption as a function of relative pressure. The hydrated casein micelle of bovine milk was chosen for this purpose.

Casein, the major protein fraction in bovine milk, exists in a unique micellar form as a calcium phosphate complex of the various monomeric casein subunits. These colloidal casein particles have intrigued many

[1] Agricultural Research Service, U. S. Department of Agriculture.

investigators, and models for the micelle structure in bovine milk have been proposed by Waugh (9, 10), Payens (11), Morr (12), and Rose (13). These models are based primarily on chemical data in addition to limited physical data from electron microscopy, with the degree of hydration substantially influencing the observed micellar size distribution (14). Furthermore, the present authors showed in a previous study of H_2O and N_2 sorption by α_{s1}-casein that only the H_2O sorption mechanism involved swelling of the protein matrix (15).

In the present study, fully hydrated casein micelles were dried by a method similar to that of Neihof, Thompson, and Dietz (7) and the changes in N_2 surface area effected by H_2O vapor sorption and desorption by these swollen particles were assessed.

MATERIALS AND METHODS[2]

Casein Preparation and Solvent Exchange. Casein was isolated by high-speed centrifugation of 1600 ml of raw skim milk at 44,330 $\times$ *g* for 1 hour in the Spinco Model L preparative ultracentrifuge. The casein pellets were washed twice by dispersing in 1 liter of distilled water, using a Ten Bröeck tissue grinder, and recentrifuging. The washed pellets were then redispersed in 550 ml H_2O and subjected to ultrasonic oscillation to prevent aggregation into large particles. Insonation was performed on 275 ml aliquots of the casein-H_2O dispersion for three 1.5-min periods using a Branson Instruments Corporation Model S75 sonifier at the maximum power output setting. The casein dispersions were kept in an ice water bath during the insonation periods to avoid overheating. The absence of large aggregates was verified by visual inspection with an optical microscope.

The water dispersion of casein, containing approximately 54 gm casein/l, was diluted with methanol at a ratio of 1 volume of H_2O to 9 of CH_3OH. During this as well as all subsequent steps involving solvent transfer ultrasonic oscillation was used to avoid aggregation. The casein settled readily from the low-density organic solvents and was therefore easily removed by low-speed centrifugation at 1200 rpm in an Internation Model V centrifuge. These pellets were washed twice with anhydrous CH_3OH redispersing, subjecting to ultrasonic oscillation, and recentrifuging.

The CH_3OH dispersion of casein, 10–15 gm/l, was diluted with a hydrocarbon solvent at a ratio of 1 volume CH_3OH to 4 of the hydrocarbon. After ultrasonic treatment of the casein and centrifugation the pellets were washed twice with the hydrocarbon solvent by similar methods and finally dispersed in the hydrocarbon before drying under high vacuum. The hydrocarbons used included pentane, hexane, and benzene of commercial purity, which were treated with CaH_2 to remove any traces of moisture. During the exchange and drying procedure, precautions were taken to avoid contact of the casein with atmospheric water vapor. After the final dispersion in the hydrocarbon the casein was immediately loaded into the glass adsorption bulbs while still wet with the organic solvent, and the residual solvent was removed under high vacuum. The sample tube loading procedure was conducted in a glove box, purged with N_2 to avoid contact with the atmosphere.

Sorption Measurements. Three- to five-gram samples of the wet casein preparations were placed in glass adsorption bulbs and outgassed under high vacuum, 10^{-6} torr, at ambient temperature for 16–24 hours. Surface area determinations were then made according to the BET method (16) using conventional volumetric procedures for measuring N_2 adsorption at -195°C in the relative pressure range $0.05 < P/P_0 < 0.35$. After the N_2 was pumped off the casein samples were thermostated at 25°C and exposed to water vapor at a controlled pres-

[2] Mention of brand or firm names does not constitute an endorsement by the Department of Agriculture over others of a similar nature not mentioned.

sure of 0.5 P_0. After H_2O was sorbed until equilibrium conditions were attained, the casein was degassed to remove the sorbed water and N_2 surface area measurements were repeated. This procedure of sorbing and desorbing H_2O followed by N_2 adsorption was continued at water vapor pressures corresponding to increments in relative pressure of 10% until measurements were made at $P/P_0 = 0.9$.

Accurate, detailed water vapor sorption data for benzene exchanged casein were determined gravimetrically with the use of the Cahn RG recording electrobalance incorporated into a glass adsorption system, by methods described elsewhere (17). Measurements were made in increments of 0.05 P/P_0 unit over the entire isotherm at 24°C through two successive sorption-desorption cycles.

RESULTS AND DISCUSSION

The effectiveness of the solvent replacement drying technique for preventing the collapse of the swollen hydrophilic structures can be seen from the data in Table I, which lists surface areas for the various dried casein preparations. Surface area values were increased by a factor of ten over that of the lyophilized caseins when the protein was dried from the straight-chain aliphatic hydrocarbons or from methanol; but when the final exchange solvent was benzene, the surface area value was much higher. The values for the two samples dried from H_2O by lyophilization show that the ultrasonic treatment did not disrupt the casein particles, thus proving it to be a safe method for dispersion and prevention of aggregation.

In his studies on cellulose fibers Merchant (8) also considered the influences of various hydrocarbons in such a drying method and reported completely opposite effects, with a value of 43 m^2/gm with benzene and progressively increasing values through cyclohexane, hexane, and pentane. The fibers dried from pentane had a surface area of 129 m^2/gm. Staudinger and Döhle (18) reported that fibers dried from either H_2O or CH_3OH had undergone so-called hornification and collapse. Mechanistic differences in the drying of cellulose fibers and of casein micelles are thus quite probable.

The loss of the expanded surface through the sorption and desorption of H_2O is apparent from the data in Fig. 1, which is a graph of the N_2 surface area values as the ordinate against the percentage of moisture sorbed by the protein during the H_2O sorption step immediately preceding the area measurement. At each H_2O sorption-desorption increment there was a decrease in surface area, but the loss became greater with an apparent change in slope in the curves at approximately 18% moisture. It seems likely that this point corresponds to a stage when

TABLE I

COMPARISON OF SURFACE AREA VALUES OF CASEIN DRIED FROM DIFFERENT SOLVENTS

Solvent	Surface area (m^2/gm)
H_2O	4.8
H_2O (insonated)	3.3
CH_3OH	41.0
C_5H_{12}	32.0
C_6H_{14}	36.1
C_6H_6	71.0

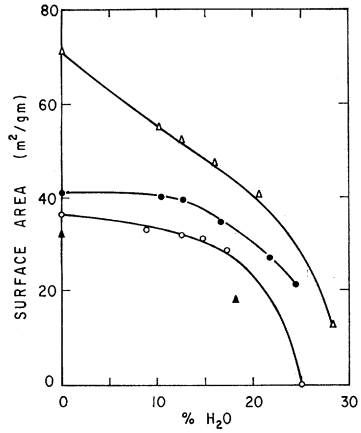

FIG. 1. Loss in surface area as influenced by H_2O sorption and desorption. The curves represent casein originally dried from ○ hexane, ● methanol, △ benzene, and ▲ pentane. No curve is drawn for pentane as only two points were measured.

the protein has become hydrated and is now more extensively swollen. Drying from such a state would cause a greater loss in surface area.

The effects of H_2O on the expanded form of the dried casein were also observed by dispersing a sample of casein dried from hexane into H_2O and freeze drying. The casein now exhibited a surface area of 1.6 m^2/gm, indicating collapse of the hydrophilic structures. The process was, however, at least partially reversible in that redispersion of the lyophilized casein in water followed by exchange through CH_3OH to C_6H_6 and redrying expanded the surface area back to 52.1 m^2/gm. This expansion of the collapsed structures was dependent on water-induced swelling. When lyophilized casein was dispersed directly into benzene or directly into methanol and dried under vacuum, surface area values of 4.5 and 6.5 m^2/gm were observed. Thus the involvement of H_2O is a prerequisite for the swelling process.

A mechanism for H_2O sorption by casein involving swelling of the dried protein is in keeping with the sorption data of Fig. 2. Hysteresis on desorption was observed during both cycles; however, the swelling process was not completely reversible. There was irreversibly bound water present after the first desorption leg. This residual moisture was termed bound in the sense that it was not desorbed on pumping at 10^{-6} torr at ambient temperature for 8 days. The lack of coincidence of the two sorption-desorption loops also indicates a difference in the sorption process in the two steps. This is entirely possible in that the macromolecular constraints on the swelling capacity may be decidely different in a protein which was dried in a manner intentionally selected to maintain a swollen state from that of a protein dried directly from a water-swollen state by vaporization of the water, as would be the case after a desorption isotherm.

In summary, contractions in the surfaces of dried casein particles have been shown to result from the direct drying of these particles from a water-swollen state. Such effects have widespread implications in biology, e.g., lyophilization has been shown (19) to cause mitochondria to lose their capacity for the coupling of electron transfer and adenosine triphosphate synthesis. Such structural changes as reported in this paper and in some of the cited studies should therefore be considered in light of the widespread usage of lyophilization to dehydrate biological and other hydrophilic materials with the intention of preserving structure.

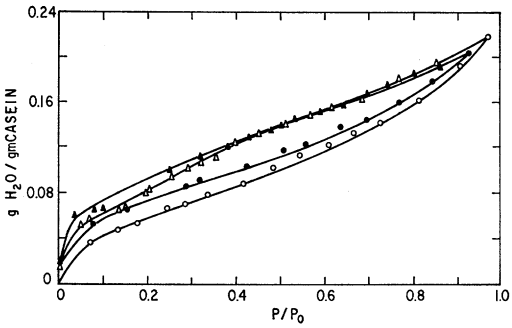

FIG. 2. Water vapor sorption data for casein solvent-exchange dried from benzene: ○, adsorption (first cycle;) △, desorption (first cycle); ●, adsorption (second cycle); ▲, desorption (second cycle).

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